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The role of visual and mechanosensory cues in structuring forward flight in *Drosophila melanogaster*

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Summary

It has long been known that many flying insects use visual cues to orient with respect to the wind and to control their groundspeed in the face of varying wind conditions. Much less explored has been the role of mechanosensory cues in orienting insects relative to the ambient air. Here we show that *Drosophila melanogaster*, magnetically tethered so as to be able to rotate about their yaw axis, are able to detect and orient into a wind, as would be experienced during forward flight. Further, this behavior is velocity dependent and is likely subserved, at least in part, by the Johnston's organs, chordotonal organs in the antennae also involved in near-field sound detection. These wind-mediated responses may help to explain how flies are able to fly forward despite visual responses that might otherwise inhibit this behavior. Expanding visual stimuli, such as are encountered during forward flight, are the most potent aversive visual cues known for *D. melanogaster* flying in a tethered paradigm. Accordingly, tethered flies strongly orient towards a focus of contraction, a problematic situation for any animal attempting to fly forward. We show in this study that wind stimuli, transduced via mechanosensory means, can compensate for the aversion to visual expansion and thus may help to explain how these animals are indeed able to maintain forward flight.

Key words: *Drosophila*, insect, flight, search, anemotaxis, flight control, Johnston's organ, mechanoreceptor, sensor fusion.

Introduction

Studies of how flying insects orient with respect to their local environment have generally emphasized the role of visual cues. Kennedy (Kennedy, 1940) first demonstrated that mosquitoes can maintain a relatively constant groundspeed, in the face of varying wind speed, via reference to the visual environment. That is, insects can compensate for variations in wind speed, or experimenter-induced movement of the visual environment, to maintain an approximately constant retinal velocity relative to the visual surround. Indeed, an animal supported by moving air has little choice but to use visual cues in order to maintain a constant velocity relative to the ground when subjected to varying wind velocity (David, 1986).

In addition to setting groundspeed, it has been suggested that visual cues are used in sensing wind direction via the detection of wind-induced sideslip (Marsh et al., 1978; Preiss and Gewecke, 1991). That is, if the longitudinal axis of the insect (and thus its presumed thrust vector) is not parallel to the wind, then the insect will drift sideways. This will result in image flow over the downward-facing ommatidia that could be decomposed into its longitudinal and transverse components. An insect attempting to fly straight upwind might try to minimize transverse flow, whereas a zigzagging moth might attempt to maintain it at a consistent value at a given wind velocity, with iteratively reversing sign (David, 1986).

The role of mechanosensory cues in flight orientation has, meanwhile, received much less attention. Though flying insects are unlikely to use mechanosensory cues to determine the direction or velocity of an externally imposed wind, it is both possible and likely that mechanosensory cues play a role in the detection of an insect's self-induced velocity relative to the ambient air, as well as yaw deviations from the direction of that thrust vector.

That insects use mechanosensory cues to control their velocity and orientation during forward flight has been strongly suggested by experiments in a taxonomically diverse range of species. Weis-Fogh first demonstrated the directional wind sensitivity of beds of trichoid sensilla on the head of locusts, *Schistocerca gregaria*, by showing that tethered insects will orient into an oncoming wind applied asymmetrically to the head (Weis-Fogh, 1948; Weis-Fogh, 1949). This led to the suggestion that an insect, flying forward, could use such a mechanism to compensate for unintended yaw. If an animal yaws relative to its direction of motion, due perhaps to bilateral asymmetry of its wing motion, the resulting skewed stimulation of the hair beds could elicit a yaw corrective maneuver. In locust swarms, for instance, an individual's yaw may average 5.2°

from its direction of flight, a deviation that might be sufficient to generate a response to mechanically registered side-slip (Baker et al., 1981).

Gewecke (Gewecke, 1970) showed that locust antennae may also function as velocity sensors because wing beat amplitude, which normally decreases as a function of wind velocity, remains elevated in individuals with immobilized antennae. Similar evidence for the role of the antennae as velocity sensors has been found in Hymenoptera (Heran, 1959), Diptera (Gewecke, 1967b), Odonata (Gewecke et al., 1974) and Lepidoptera (Gewecke and Niehaus, 1981; Niehaus, 1981), although not in the wasp *Paravespula vulgaris* (Brandstatter, 1990). Arbas (Arbas, 1986) showed that the antennae may also play a role as directional wind sensors in *Schistocerca gregaria*, complementing the hair plates, and data from blowflies suggest that this may be true across insect orders (Gewecke, 1967b; Schneider, 1953). Recently, it has also been suggested that the antennae may detect Coriolis forces during rotational maneuvers in *Manduca sexta*, playing an additional, potentially important role in stabilizing flight (Sane et al., 2007). In this study, we examined the role of the antennae and mechanosensory cues in orienting the flight of *D. melanogaster*, specifically in the context of a recently discovered, and apparently paradoxical, visual flight control mechanism.

Experiments in freely flying *Drosophila* (Tammero and Dickinson, 2002b) have indicated that expanding visual stimuli are extremely potent at eliciting avoidance responses. This is an intuitive result inasmuch as it may function in collision avoidance, turning a fly away, for example, from a rapidly expanding image of a tree branch. A tethered fly in a closed loop paradigm presented with a symmetrically drifting visual pattern consisting of a focus of expansion (FOE) and an opposite focus of contraction (FOC), exhibits behavior consistent with these results. In this case, flies turn away from the FOE and steer instead towards the FOC (Bender and Dickinson, 2006; Tammero and Dickinson, 2002a). In the artificial environment of a visual flight arena, this behavior is perhaps not surprising, but a freely flying fly that does not orient towards a FOE could find itself in the curious situation of being unable to fly forward.

Recent work by Reiser (Reiser, 2007) has indicated that the strength of the expansion avoidance response is a function of the temporal frequency of expansion; the rate at which a periodic pattern of light and dark stripes moves across a point on the fly's retina as it moves from the FOE towards the FOC. Whereas flies orient towards the FOC at high rates of expansion, orientation towards the FOE is increasingly favored as the expansion rate is reduced (Reiser, 2007). Depending upon the temporal frequencies experienced by flies in the real world, this velocity dependence could partially explain how flies are indeed able to fly forward under many natural conditions, yet turn away under other conditions, such as when they approach an obstacle. Another possibility is that the fly uses additional sensory cues to stabilize forward motion. The perception of a headwind created by self-motion, for example, may be necessary to sustain forward flight.

It thus seems reasonable to ask whether *D. melanogaster* relies on mechanosensory feedback in controlling its orientation during forward flight. To answer this question, we quantified orientation in loosely tethered flies (allowing them to rotate about their yaw axis) in a wind tunnel, across a range of wind velocities. We then explored the degree to which this is a passive aerodynamic or active behavioral response, and assessed the role of the Johnston's organs (JOs), a paired set of antennal chordotonal organs, in mediating the behavior. In *Drosophila*, the antennae have long been known to be involved in the detection of near-field sounds during courtship displays (Ewing, 1983), with the JOs detecting relative displacements of the pedicel and funiculus (Eberl et al., 2000; Ewing, 1978; Gopfert and Robert, 2002). However, the role of JOs in wind detection, and that of the antennae generally, has heretofore been unexplored in *D. melanogaster*.

To test whether mechanosensory feedback may also help to account for the tolerance of visual expansion that must accompany forward flight, we presented flies with visually expanding stimuli at several temporal frequencies paired with a range of wind velocities. Our results suggest that a strong mechanosensory orientation response, such as that generated by self-motion in forward flight, may indeed help to explain free-flight behavior by overcoming the repulsive effects of strong visual expansion.

Materials and methods

Animals

All experiments were performed on flies descended from a population of 200 wild-caught female *Drosophila melanogaster* (Meigen). Females aged 3–5 days post-eclosion were deprived of food, but not water, for 4–6 h prior to experimentation.

Flight arena

For experiments, the blunt end of a 50 μm diameter steel pin was glued to the anterior of the fly's notum. The pin and fly were then suspended vertically between two magnets such that the pin's sharp end rested in a V-aperture sapphire bearing that was glued to the magnet positioned above the fly (Fig. 1). The pin was thus aligned parallel to the magnetic field lines, allowing the fly to rotate around its functional yaw axis with minimal friction. This design is based on that described in Bender and Dickinson (Bender and Dickinson, 2006), except that here the magnet located beneath the fly consisted of a stack of five 3.8 cm diameter ring magnets. A ring of 880 nm LEDs around the lower magnet provided illumination for the IR visualization system used to track fly orientation.

Tethered flies were tested in a cylindrical arena, composed of green LEDs, with a height of 0.095 m and a diameter of 0.203 m (Fig. 1). The arena had a circumference and height of 160 and 24 LEDs, respectively, with 24 columns removed at the up- and downwind ends to allow wind to flow through the arena, impinging on the tethered flies. A detailed technical description of the modular display system, which was controlled by a dedicated controller board operating under the command of a PC, is provided in Reiser and Dickinson (Reiser and Dickinson, 2007).

Wind tunnel

The visual arena and magnetic tether were placed in an open circuit, closed throat wind tunnel with an acrylic working section with a width and height of 0.305 m. This tunnel was

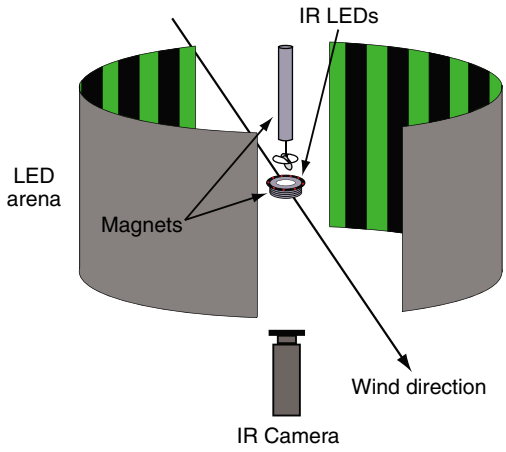

Fig. 1. A schematic view of the visual arena. The arena had a circumference of 160 rows and a height of 24 rows of LEDs with 24 columns removed at the up- and downwind ends to allow smooth airflow over the fly. Flies were glued to a steel pin, positioned between two magnets, allowing rotation around the functional yaw axis. A camera positioned below the tunnel visualized the fly at 100 Hz through a hole in the center of a set of ring magnets.

identical to the one described in Budick and Dickinson (Budick and Dickinson, 2006) except that the length of the working section was 0.914 m, the floor was transparent and the walls were covered with white paper. In addition, no lighting, besides that from the visual arena and the IR LEDs, was provided, though the room was not completely dark due to the presence of a computer monitor. Flow through the arena appeared laminar when visualized with a smoke plume. Wind velocity was controlled by custom-made software running on a PC, which regulated the tunnel's motor speed via a voltage signal to the motor controller. To change tunnel speed, the tunnel motor followed a constant acceleration trajectory. Wind velocity was validated by smoke visualization, an ultrasonic anemometer and a thermistor-based anemometer. Experiments were performed between 23.5 and 25°C.

Data acquisition

The fly visualization system consisted of an IR camera, positioned under the floor of the acrylic tunnel, that directly visualized the fly through the hole in the center of the circular magnets at a frame rate of 100 Hz (for details, see Bender and Dickinson, 2006). The same PC that controlled the visual arena and tunnel velocity also recorded the tunnel's actual motor speed and the position of the visual stimulus at 12 Hz, as well as the fly orientation at 100 Hz.

Experiment 1

To test orientation as a function of wind velocity, 33 flies were randomly presented with wind at 0, 0.2, 0.4, 0.6, 0.8 and 1.0 m s^{-1}, each wind velocity being presented for 10 s with the visual arena turned off. Between trials the wind was stopped and flies were presented with an open loop visual stimulus for 10 s, consisting of an expanding pattern of vertical stripes with a spatial frequency of 36° and with the FOC at the downwind end of the arena. This stimulus realigned the flies to a downwind orientation between trials, thereby standardizing their initial orientation. Because fly orientations tended to remain stable over the second half of each 10 s trial, data from only the first 5 s of each trial were analyzed.

Experiment 2

To test for the effects of passive, wind-induced orientation, flies were divided into three experimental groups. We froze one group for 1 h before tethering them (n=20) while we similarly froze a second group and then clipped their wings at the hinge prior to tethering (n=18). The third, control group (n=23) was tethered live. Flies were then exposed, in the dark, to wind velocities of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 m s^{-1} with trial lengths of 5 s. Unlike experiment 1, flies were not visually reoriented between trials (because dead flies would be unable to visually reorient) and instead trials were interspersed with 5 s periods of darkness with no wind.

Experiment 3

To test the role of the JOs in wind-mediated orientation, a small drop of UV-sensitive glue was placed at the junction between the pedicel and the funiculus, either unilaterally or bilaterally, and illuminated with a UV lamp for 20 s prior to tethering the fly. This had the effect of deafferentating the JOs, which are located in the pedicel and are sensitive to relative deflections of these two antennal segments. Flies subjected to ablation of the antennae at the level of this joint did not fly robustly enough in our apparatus for quantitative analysis. Four groups of flies were tested, those with neither antenna glued (n=27), and those with the right (n=26), left (n=29) or both (n=32) antennae glued. Flies were then tested at 0, 0.2 and 1.0 m s^{-1} wind velocities in 10 s trials after which they were again visually reoriented by the same expanding stimulus used in experiment 1, with no wind, for 10 s. As in experiment 1, fly orientation tended to be stable over the second half of each 10 s trial, so data from only the first half of each trial were analyzed.

Experiment 4

To test the relative contributions of wind and visual stimuli to fly orientation, 26 flies were subjected to 39 different combinations of wind velocity, visual expansion rate and azimuthal position of the FOE. The expanding visual pattern consisted of the same vertical stripes used for realignment between trials in experiments 1 and 3. Flies were tested at wind velocities of 0, 0.2 and 0.6 m s^{-1}. The expansion pattern was composed of two half-fields consisting of a square-wave pattern (spatial frequency of 36°), moving at angular speeds of 9, 36, and 180° s^{-1}, corresponding to temporal frequencies of 0.25, 1.0 and 5 Hz. The FOE was positioned at 0° (upwind), +90°, –90° or 180° (downwind). Every wind velocity was paired with every expansion rate and FOE position for a total of 36 treatments. In addition, the flies were also tested at all three wind velocities in the absence of any visual stimuli for a total of 39 treatments per fly with treatments presented in random order. Trials lasted 5 s and the flies were not visually reoriented in the 5 s intervals between trials, during which the visual display was turned off, in order to avoid biasing their initial orientations.

Analysis

In order to quantify orientation behavior, we defined an orientation response metric as follows. The mean circular orientation was calculated over the first 100 ms (initial orientation) and over the final 2 s (final orientation) of each 5 s analysis period. Subtracting the absolute value of the final orientation from the absolute value of the initial one yielded the orientation response, where a positive value indicates that the final orientation is more closely aligned with upwind than the respective initial orientation. The magnitude of the response is limited by the initial orientation (which was biased towards 180° in experiments where flies were visually reoriented between trials). Because of the scatter in initial orientations, we devised a second metric, the response index, that is independent of initial orientation. This metric is calculated as (90°−|final orientation|)/90°. A response index of +1 corresponds to a turn that maximally orients the fly towards upwind, –1 to a turn that orients the fly downwind, and 0 to a turn that results in a final orientation of ±90°. In all experiments, any fly that stopped flying during the recording period was excluded from analysis and a single set of trials was analyzed for each fly. All statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA) or JMP (SAS Institute Inc., Cary, NC, USA).

Results

Experiment 1

Flies responded to a wind stimulus by orienting into the oncoming wind (Fig. 2A). Because of the scatter in initial orientation, we visualized the magnitude of the orientation response by plotting it as a function of the absolute value of the initial orientation (Fig. 2B,C). The response index, which was independent of initial orientation (Fig. 2D), was significantly different from baseline (no wind) at all wind velocities from 0.2 to 1.0 m s^{-1} (0.2 m s^{-1}: N=33, Z=–4.08, P<0.001; 0.4 m s^{-1}: N=33, Z=–3.83, P<0.001; 0.6 m s^{-1}: N=33, Z=–4.32, P<0.001; 0.8 m s^{-1}: N=33, Z=–4.58, P<0.001; 1.0 m s^{-1}: N=33, Z=–4.78, P<0.001; Wilcoxon signed ranks test) with a significant dependence on wind velocity between 0.2 and 1.0 m s^{-1} when fly identity was controlled via its inclusion as a nominal variable in a multiple regression (b=0.28, t=5.49, P<0.001; Fig. 2E).

Experiment 2

It was possible that the orientation response was in part due to passive aerodynamic effects of wind on the fly. We therefore compared the responses of live tethered flies with those of freshly killed flies with their wings extended. To further parse the effects of wind on the body and wings, we removed the

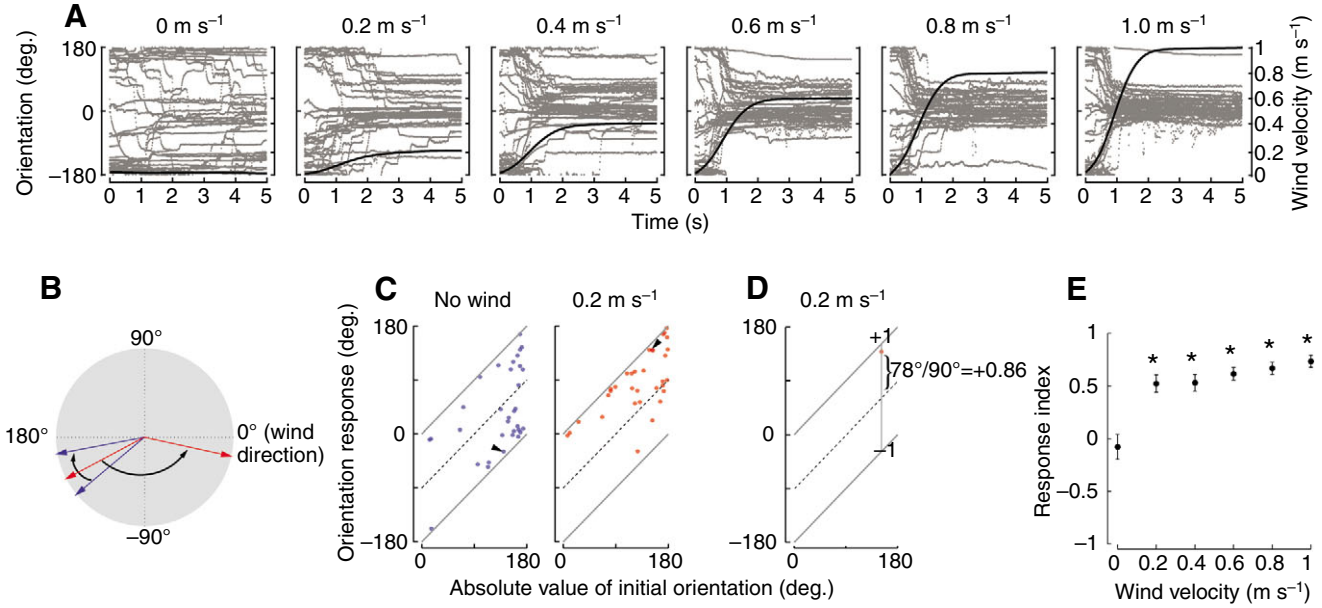

Fig. 2. Loosely tethered *D. melanogaster* orient upwind. (A) Flies randomly presented with wind velocities between 0 and 1.0 m s^{-1} orient progressively more tightly around 0° (upwind) with increasing wind velocity. The heavy black lines indicate the time course of wind velocity. (B) Orientation changes were quantified by an orientation response metric. The mean circular orientation was calculated over the first 100 ms (initial orientation) and the final 2 s (final orientation) of each trial. Orientation response is then given by |initial orientation|–|final orientation|. For example, a fly responded to the onset of a 0.2 m s^{-1} wind by turning from an initial angle of –150° to a final angle of –12°; an orientation response of 138° (red arrows). In the absence of wind, the same fly turned from –140° to –169°; an orientation response of –29° (blue arrows). (C) Plotting orientation response as a function of the absolute value of the initial orientation provides evidence for orientation to wind (arrowheads indicate the fly whose responses are shown in B). Responses falling along the upper solid line represent perfect upwind orientation, while those along the lower line indicate responses diametric from upwind. (D) A second metric, the response index, quantified responses independently of initial orientation. The response index was calculated as (90°−|final orientation|)/90° where +1 indicates a response with a final orientation of 0°, –1 corresponds to a final orientation of 180° and 0 indicates a response with a final orientation of ±90°. The response index is thus (90°–12°)/90°=0.86 for the fly represented by the red arrows in B (dashed line indicates response index=0). (E) Response index varied significantly with wind velocity between 0.2 and 1.0 m s^{-1}, with responses at all velocities being significantly greater than in no wind.

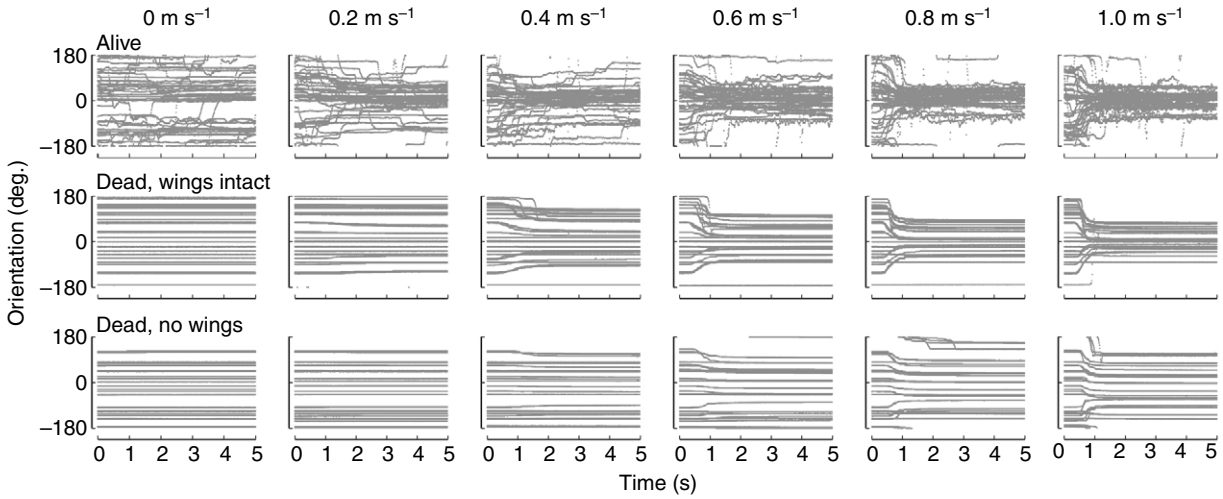

Fig. 3. A passive aerodynamic response is apparent with increasing wind velocity. Dead flies with their wings intact and, to a lesser extent, dead flies without wings also evinced an orientation response to wind onset.

wings from a subset of the dead flies. Visual examination of the time course of orientation in these three groups clearly indicates that there was a substantial passive response to the wind, particularly at high wind velocity and particularly in flies with intact wings (Fig. 3). Orientation changes in dead flies rapidly followed the start of the wind stimulus and remained unchanged for the duration of the trial. The fact that most dead flies did not reach a perfect upwind orientation could have been due to a variety of factors, including friction between the pin and sapphire bearing, small irregularities in the magnetic field, or non-uniform tethering. However, in almost all cases the wind reoriented the flies in a more upwind direction. Plotting the orientation response as a function of the absolute value of the initial orientation emphasizes the fact that the passive response of dead flies becomes more pronounced at velocities greater than 0.4 m s^{-1} (Fig. 4). Nevertheless, live flies had significantly higher response indices than dead flies with intact wings at velocities less than 0.8 m s^{-1} (and these were nearly significant at 0.8 m s^{-1}; 0.2 m s^{-1}: t=3.11, d.f.=36, P<0.005; 0.4 m s^{-1}: t=2.24, d.f.=36, P<0.05; 0.6 m s^{-1}: t=2.28, d.f.=36, P<0.05; 0.8 m s^{-1}: t=1.58, d.f.=36, P=0.06; 1.0 m s^{-1}: t=1.37, d.f.=36, P=0.09; one-tailed, homoscedastic t-tests; Fig. 5A).

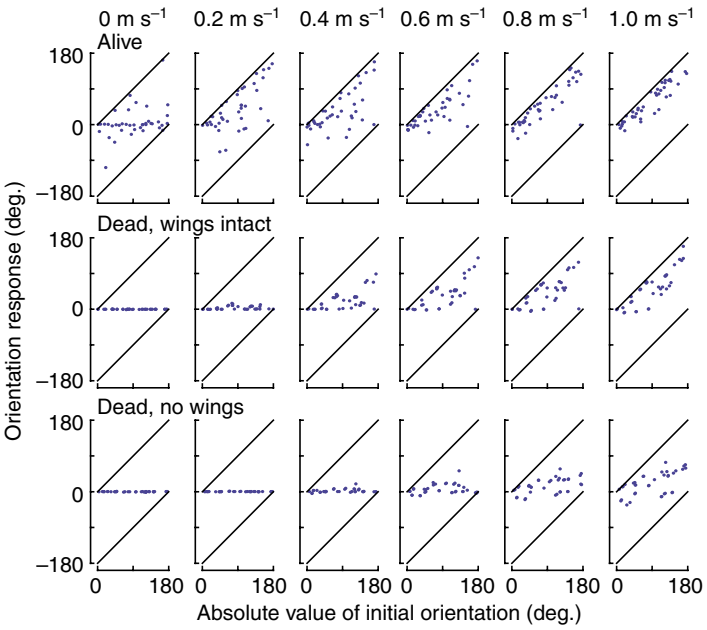

Fig. 4. Orientation responses were much more pronounced in live flies, particularly at low wind velocity. Plotting orientation response as a function of |initial orientation| makes it apparent that responses in dead flies without wings were quite small, even at high wind velocity, while dead flies with intact wings manifested moderately strong orientation, especially at the highest wind velocities.

To offer a rough quantification of the passive orientation effect of the wings, we subtracted the mean response index of dead, wingless flies, at each wind velocity, from the mean response index in dead, winged flies. Dividing this quantity by the response index in dead, winged flies yields the percentage of the response that can be attributed to wing effects alone, assuming that the aerodynamic effects on the body and wings are purely additive (Fig. 5B). We similarly calculated the contribution of the active, behavioral response, via comparison of the responses of live and dead, winged flies. Wings accounted for a declining fraction of the passive response, from nearly 100% at 0.2 m s^{-1} to 61% at 1.0 m s^{-1}, whereas the behavioral response accounted for 85% of the response of live, winged flies at 0.2 m s^{-1}, declining to 14% at 1.0 m s^{-1}. However, it should be noted that because this metric involves a ratio, the calculation may be subject to error when the denominator is small (i.e. among the response indices for dead flies at low wind velocities). Further, the dead flies' wings were extended either laterally or dorsally and so represented a 'snapshot' of the conformations that normally occur during the full wing stroke cycle. The aerodynamic influence of wind on two stationary wings is, therefore, only a very rough

approximation of the effects expected on two continuously flapping ones.

To determine whether body saccades played a substantial role in these responses, we identified saccades using the same algorithm as in Bender and Dickinson (Bender and Dickinson, 2006). Saccades were defined as turns of amplitude greater than 15° with peak angular velocities exceeding 300° s^{-1} (Fig. 6A,B). In the absence of wind, spontaneous saccades by live flies were distributed relatively uniformly throughout the trial, and did not tend to orient the flies towards the upwind end of the tunnel (Fig. 6C). In the presence of wind, saccades tended to occur near the onset of the trial and usually oriented flies towards, rather than away from, upwind. Such events are visible in the raw traces in Fig. 4. The data traces from dead flies rarely fulfilled the saccade criteria, further supporting the interpretation that the upwind orientation of flies includes an active behavioral component.

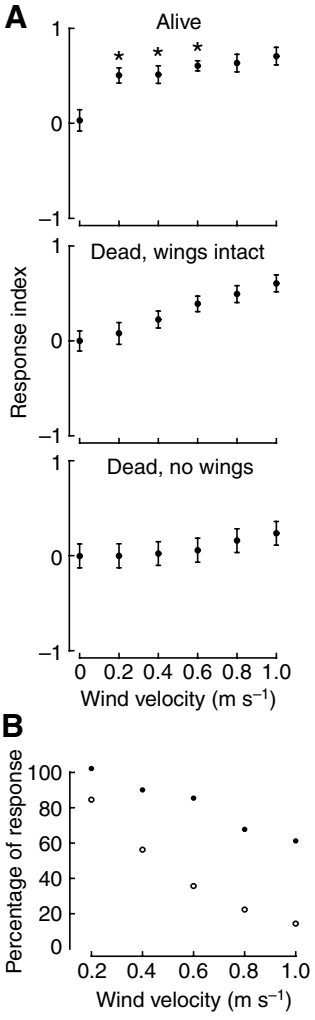

Fig. 5. Response index scores permit a decomposition of the active and passive responses. (A) Response index scores were significantly higher in live flies (asterisks) than in dead, winged flies at velocities below 0.8 m s^{-1}. (B) The percentage of the response attributable to the aerodynamic effects of wings was quantified by subtracting the mean response index for dead wingless flies from the corresponding values for dead, winged flies and dividing by the mean, dead, winged response (filled circles). The effect of the live behavioral response was similarly quantified from the responses of live flies and dead, winged flies (open circles).

Experiment 3

Because the JOs have been implicated in the detection of wind direction in a variety of insects, we tested the effects of unilateral and bilateral deafferentation of these chordotonal organs by using glue to fix their relative orientation (see Materials and methods). Because *D. melanogaster* may lack the single campaniform sensillum present at this junction in some other Diptera (Miller, 1950) (D. Eberl, personal communication) it is likely that resulting behavioral deficits can be ascribed to a loss of directional sensitivity in the JOs.

Examination of the raw orientation traces indicates that orientation at low wind velocity was severely reduced in the case of bilaterally glued flies and, to a lesser extent, in unilaterally glued flies (Fig. 7A). At high wind velocity, orientation was impaired to a much slighter degree, consistent with the participation of a passive aerodynamic response at elevated wind speeds. Flies with unaltered antennae responded significantly better than baseline (no wind) at both wind velocities (Fig. 7B; 0.2 m s^{-1}: t=–4.84, d.f.=26, P<0.001; 1.0 m s^{-1}: t=–5.26, d.f.=26, P<0.001). At 0.2 m s^{-1}, bilaterally glued (t=0.24, d.f.=31, P=0.49), left antenna glued (t=–0.35, d.f.=28, P=0.37) and right antenna glued (t=–2.77, d.f.=25, P=0.055) flies did not orient significantly better than baseline, although the right antenna glued response was nearly significant. At 1.0 m s^{-1}, bilaterally glued (t=–3.65, d.f.=31, P<0.001), right antenna glued (t=–4.04, d.f.=25, P<0.001) and left antenna glued (t=–3.61, d.f.=28, P<0.001) flies all performed significantly better than baseline. There was an apparent asymmetry in the turning responses of unilaterally glued flies, as right antenna glued and left antenna glued flies turned from 180° and –180°, respectively, towards 0°. In both cases, it appeared as though flies tended to orient asymmetrically during the visual reorientation period between trials, taking the shortest path from their initial orientations towards 0°. It is not apparent that their final orientations were similarly asymmetric.

To quantify the relative contributions of the JOs to the active behavioral response, we performed an analysis analogous to that of experiment 2, wherein we estimated the active and passive contributions to the total orientation response (Fig. 5B). In this case, we calculated the fraction of the unilaterally glued response attributable to a single antenna by subtracting the mean response index in bilaterally glued flies, at each wind velocity, from the mean responses of the unilaterally glued flies. Dividing this difference by the response index of the unilaterally glued flies yielded the contribution of a single antenna to their response: 73% and 4% at 0.2 and 1.0 m s^{-1}, respectively (Fig. 7C). We quantified the additional effect of the second antenna by subtracting the mean responses of unilaterally glued flies from those of unaltered, control flies, yielding contributions of 52% and 20% at 0.2 and 1.0 m s^{-1}, respectively.

Experiment 4

To test the relative contributions of visual and wind stimuli in determining the orientation of tethered flies, we presented

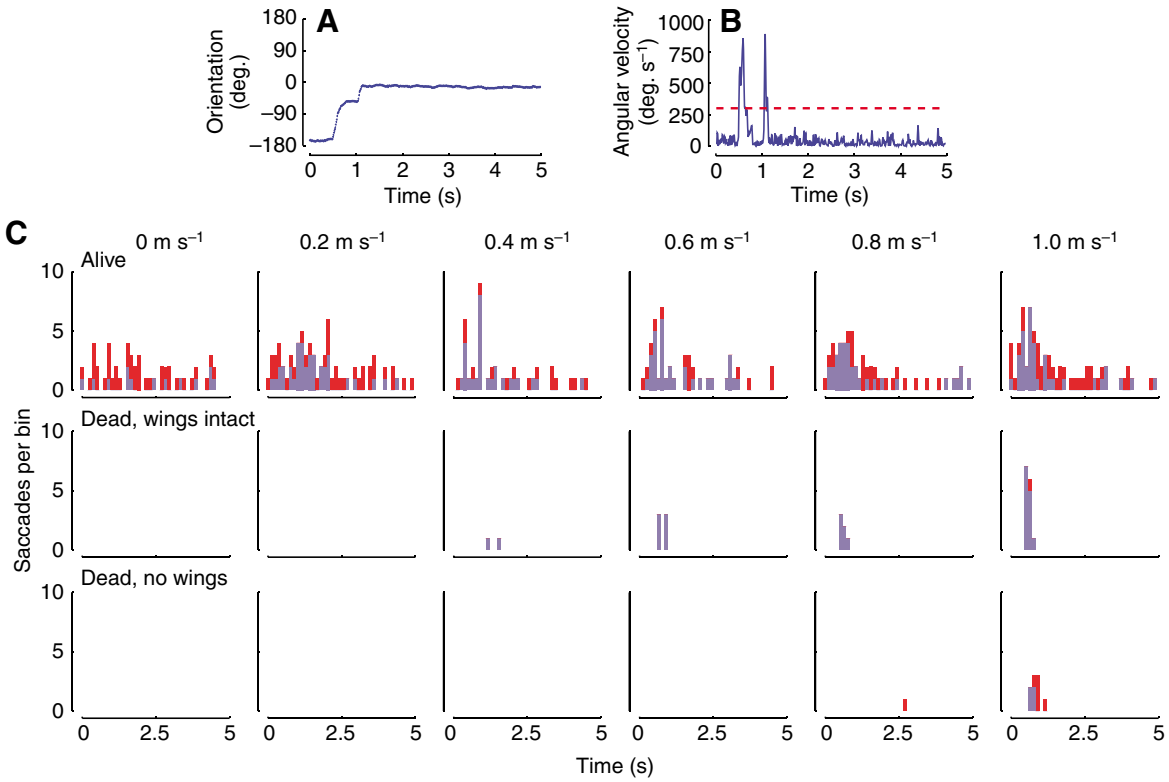

Fig. 6. Saccades play a role in the active behavioral response. Saccades were quantified as turns with magnitudes greater than 15° and angular velocities exceeding 300° s^{-1}. (A) An orientation trace from a fly orienting in a 0.6 m s^{-1} wind manifests two rapid turns. (B) An angular velocity trace of the same data as in A, illustrating the spikes in angular velocity that characterize saccades. (C) In live flies, spontaneous saccades (i.e. those exhibited in the absence of wind) were distributed throughout the trial (note that the histograms are stacked). Furthermore, those that improved the flies' orientation relative to upwind (blue bars) did not predominate compared with those that turned the flies away from upwind (red bars). In the presence of wind, saccades tended to cluster near the onset of the wind stimulus and also tended to improve the orientation relative to upwind. In dead flies, saccades were very rare under all conditions.

them with combinations of multiple wind velocities, visual expansion rates and azimuthal orientations of the expansion pattern. To quantify the relative contributions of the wind and visual stimuli to orientation, we calculated a preference index as follows (Fig. 8). We divided the number of instantaneous orientation vectors (the fly orientation in each frame) that fell within ±45° of upwind (0°) over the 5 s trial period by the total number of vectors that fell within ±45° of the FOC and ±45° of upwind. The preference index thus ranged from 1 (perfect wind orientation) to 0 (perfect FOC orientation). A preference index could only be calculated for FOC locations at ±90° and 180° because its computation requires a minimum 90° offset between the FOC and upwind. In the absence of a visual stimulus, flies oriented into an oncoming wind (Fig. 9, bottom row), and did so with increasing fidelity at higher wind velocities as described earlier (Fig. 2). When paired with a visual stimulus, preference indices increased with wind velocity within a given expansion rate and across FOC locations (Fig. 9). Between expansion rates and within wind velocities, the rate of visual expansion generally had the opposite effect and tended to orient flies in the direction of the FOC (Fig. 9). When both the wind and visual stimuli favored upwind orientation, the flies tended to orient rather uniformly upwind, although orientation did seem to decline when wind was combined with the highest rate of visual expansion. The competition between these stimuli can be seen most clearly in rows two and three of columns four to nine of Fig. 9. At both expansion rates, fly orientation is more strongly influenced by the wind as wind velocity increases.

The flies' orientation response can be interpreted as a multivariate function of wind velocity, expansion rate and the azimuthal position of the FOC. To quantify the relative effects of these three cues, we performed a multiple linear regression of the preference index on these three predictors, while controlling for fly identity by including it as a nominal variable. The results, shown in Table 1, indicate that all three predictors contributed significantly to a combined model with standard partial regression coefficients of similar magnitudes. Fly orientation in this paradigm can thus be described as a trade-off between an attraction towards upwind (forward flight) orientation and an avoidance of expanding visual stimuli.

Table 1. *Multiple regression of preference index on visual expansion rate, wind velocity and FOC location*

	t	β	*P*	R^2
Expansion rate	–10.70	–0.325	<0.001	0.33
Wind velocity	14.22	0.431	<0.001	
FOC location	–6.73	–0.204	<0.001	

FOC, focus of contraction.

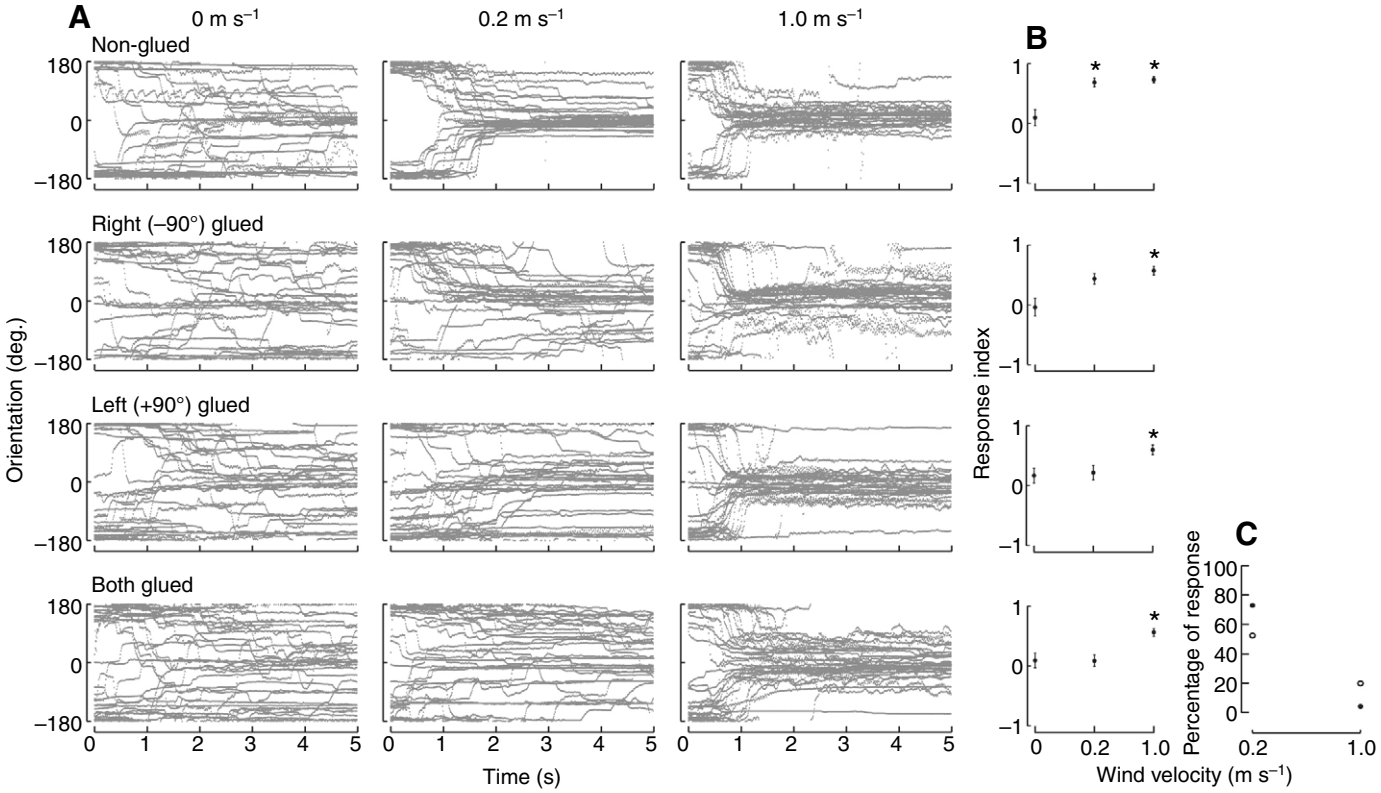

Fig. 7. Antennal immobilization greatly reduces the orientation response. (A) Orientation traces from flies that had their antennae glued, either unilaterally or bilaterally, indicate a decrement in orientation ability. (B) At 0.2 m s^{-1}, orientation was not significantly different from baseline in bilaterally glued flies (t=0.24, d.f.=31, P=0.49), or in flies with the right (t=–2.77, d.f.=25, P=0.055) or left (t=–0.35, d.f.=28, P=0.37) antennae unilaterally glued. Control flies, however, did orient significantly better than baseline at the lower velocity (t=–4.84, d.f.=26, P<0.001). At 1.0 m s^{-1}, all groups oriented significantly better than baseline (non-glued: t=–5.26, d.f.=26, P<0.001; right antenna glued: t=–4.04, d.f.=25, P<0.001; left antenna glued: t=–3.61, d.f.=28, P<0.001; both antennae glued: t=–3.65, d.f.=31, P<0.001). (C) The percentage of the response attributable to a single JO was quantified by subtracting the mean response index for bilaterally glued flies from the corresponding mean values for flies with one antenna glued and dividing by the unilaterally glued response (filled circles). The effect of the second JO was similarly calculated from the responses of non-glued flies and the mean unilaterally glued responses (open circles).

Further, as the FOC moved from a crosswind position (±90° relative to the direction of an oncoming wind) to a position directly downwind, wind fixation declined as flies increasingly chose to orient towards the FOC, rather than adopting a compromise orientation. Since the visual arena contained gaps at its up- and downwind ends, this result indicates that the presence of the pole itself was not necessary in order to evoke a visual response.

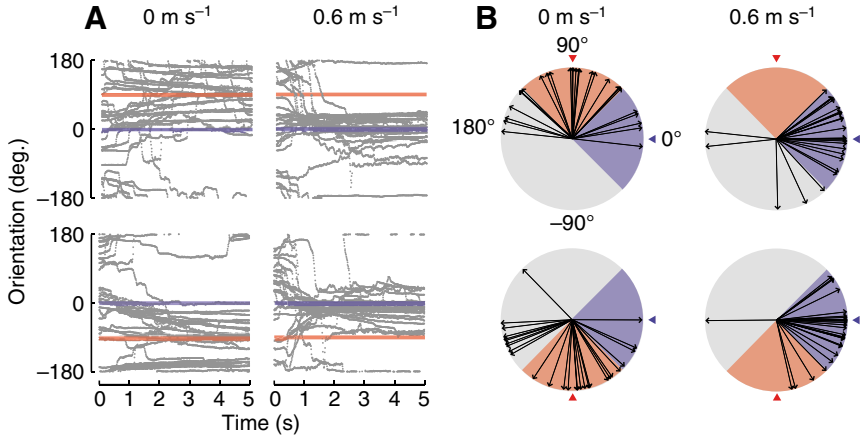

Fig. 8. Flight orientation was quantified in response to competing wind and visual stimuli. Flies were presented with 39 different combinations of wind velocity, expansion rate and orientation of the expansion pattern. (A) Exemplar responses are shown to a striped pattern expanding at a temporal frequency of 1.0·Hz, from a focus of expansion (FOE) at ±90°, in the absence and presence of a 0.6 m s^{-1} wind [location of the focus of contraction (FOC) is indicated by the red bar, orientation favored by wind is indicated by the blue bar]. (B) To quantify the preference for the visual or wind stimulus, the number of instantaneous heading vectors in each trial that fell within ±45° of 0° (blue shaded area) was divided by the total number falling within ±45° of 0° and ±45° of the FOC (red shaded area). This yielded a preference index between 1 (wind preferred) and 0 (FOC preferred). In B, the arrows represent the mean orientation vectors of each fly in A over the full 5 s trial.

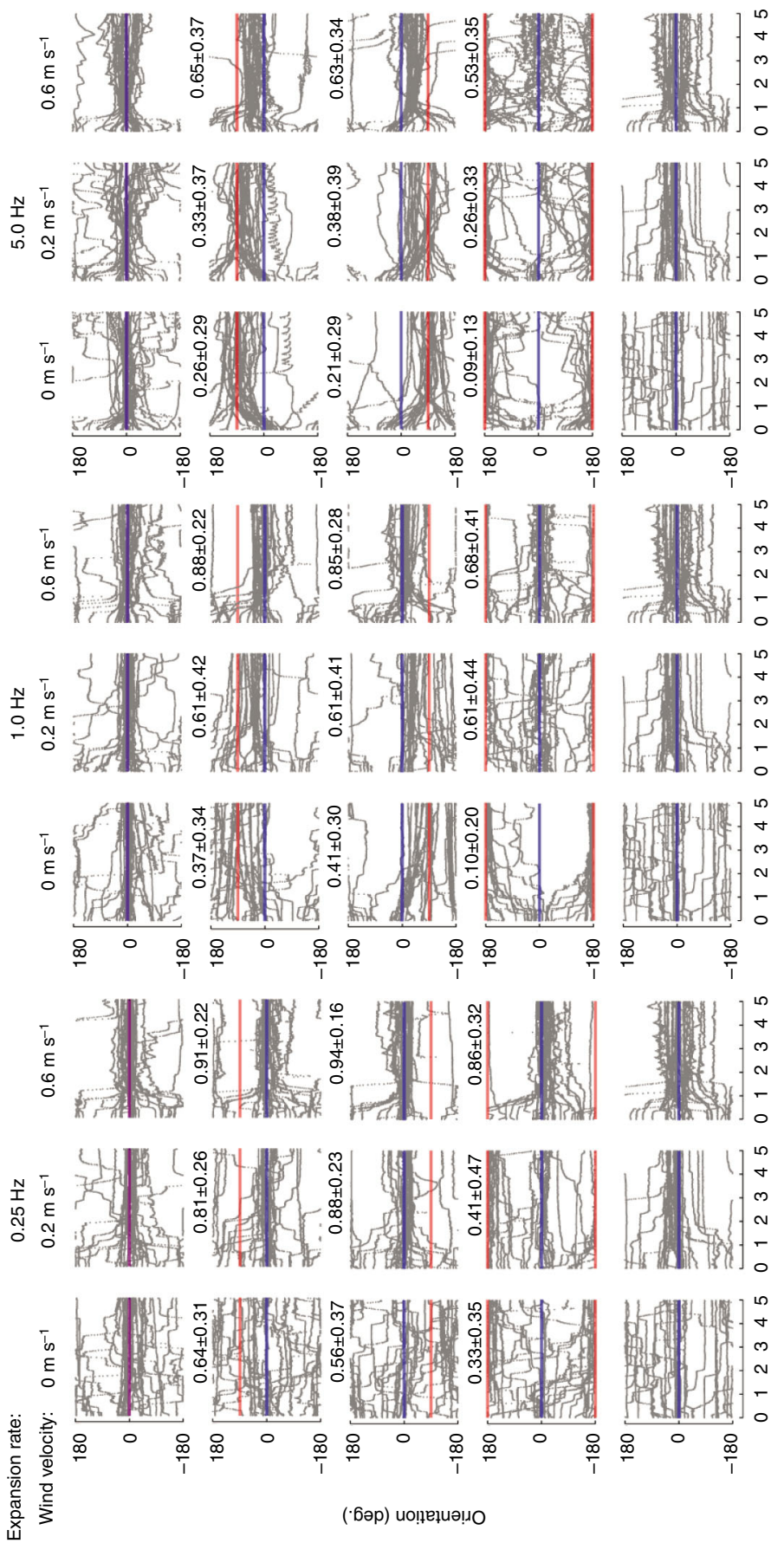

Fig. 9. Both the visual and wind stimuli shaped fly orientation. The orientation predicted by a preference for wind is indicated by the blue bars and that predicted by FOC orientation is indicated by the red bars. Each row represents a change in the location of the FOC with the bottom row corresponding to the orientation behavior measured while the visual arena was dark. Numerical values above and to the right of the labeled panels are mean preference indices ±s.d. Orientation could, in general, be described as a compromise between the two competing stimuli with orientation being increasingly biased towards the wind with increasing wind velocity, and increasingly favoring the FOC with increasing expansion velocity.

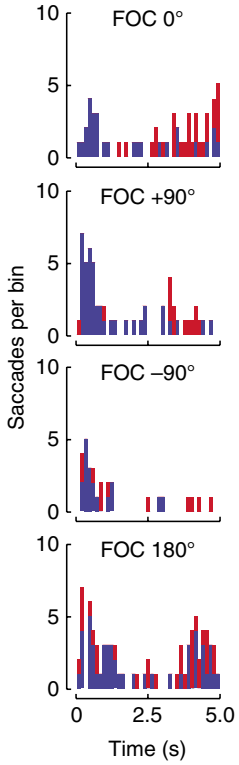

Fig. 10. Saccades were employed, as with the wind stimuli (Fig. 6) to orient flies towards an attractive visual stimulus. A visual pattern expanding at 5 Hz in the absence of a wind stimulus tended to elicit saccades that oriented flies towards (blue bars) rather than away from the FOC (red bars), particularly at the beginning of the trial (note that histograms are stacked). Flies also exhibited a large number of saccades towards the end of trials with expansion rates of 5 Hz, particularly when the FOC was at either the up- or the downwind ends of the arena.

Saccades, as indicated in experiment 2, were a conspicuous component of the responses of live flies to the onset of wind. In this experiment, saccades similarly served to orient flies in the direction of the FOC in cases where it was the preferred stimulus (Fig. 10). Interestingly, saccades were also common towards the end of trials with the highest visual expansion rate when the FOC was at 0° or 180°, and thus had the effect of turning flies away from the previously attractive FOC. That flies tended to reorient towards the end of these trials is apparent in the raw orientation traces at 5.0 Hz, yielding significantly lower fixation indices when the FOC was positioned at 180° as compared to ±90° (N_1=54, N_2=27, U=507, P<0.03; Mann-Whitney U-test). Aside from their timing, saccade dynamics were very similar across trials (data not shown).

Discussion

In this study, we have shown that *D. melanogaster*, when magnetically tethered so that they are able to freely rotate around their yaw axis, tend to orient into an oncoming wind. This stimulus is analogous to one that would be experienced during forward flight due to self motion, and the fidelity of orientation is positively correlated with wind velocity within a range that a fly is likely to encounter in free-flight (Budick and Dickinson, 2006; Tammero and Dickinson, 2002b). This orientation occurs rapidly in response to stimulus onset and is sustained over a 5 s stimulus period. This result is consistent with observations of orientation into a headwind in tethered *Schistocerca gregaria* (Weis-Fogh, 1948; Weis-Fogh, 1949) and *Locusta migratoria* (Gewecke and Philippen, 1978).

A fraction of the orientation response resulted from a passive, 'wind vane' effect, especially at high wind velocity. However, it is difficult to assess the extent to which such a passive response may also occur in free-flight. The magnitude of the passive effect is determined by the relative position of the fly's center of mass, which is likely to reside near the anterior end of the abdomen in a gravid female (W. Dickson, personal communication), and the center of pressure acting on the body and wings. Flies in this experiment were tethered at the front end of the thorax, anterior to the center of mass, situating a greater fraction of the fly's body and wing area posterior to the axis of rotation. Any passive force observed here is therefore likely to be exaggerated relative to free-flight. An additional uncertainty in estimating the magnitude of the passive response is in determining the average aerodynamic effect of two flapping wings, which is only roughly approximated by two stationary ones. Thus, whereas the passive response in this experiment acted complementarily to the active one, it is not possible, without further experimentation, to determine whether a similar force acts in free-flight. It does seem most likely, though, that a free-flight passive response, if it exists, will be of smaller magnitude than that observed here based on the expected location of the center of mass.

Regardless of the passive effects of wind-induced orientation in freely flying flies, the passive response is supplemented by a substantial behavioral response that explains a progressively greater proportion of the total response as wind velocity decreases. Inasmuch as flies may rarely reach the high air speeds where the passive response becomes more prominent (Budick and Dickinson, 2006; Tammero and Dickinson, 2002b), this phenomenon may have a relatively small effect in free-flight. Further, when visual and wind stimuli were presented in opposition to each other, flies were capable of choosing an orientation diametric from the wind direction, again indicating that the passive response cannot explain the observed orientation responses. Finally, the behavioral response involved high angular velocity saccades, a phenomenon absent from the passive response, but a conspicuous feature of free-flight behavior (Frye et al., 2003; Tammero and Dickinson, 2002b).

Dissecting the transduction pathway by which wind stimuli are detected has been made difficult by the presence of multiple mechanoreceptors that are potentially sensitive to the relevant stimuli. For example, in *Locusta migratoria*, the JOs, a ring of 70 campaniform sensilla, and an additional chordotonal organ are all sensitive to displacements of the flagellum relative to the pedicel (Gewecke, 1972), with the campaniform sensilla playing an integral role in directional sensitivity (Gewecke and Heinzel, 1979). In *C. erythrocephala*, the JOs, together with a single campaniform sensillum, respond to passive movements of the basal annulus of the flagellum, known as the funiculus in Diptera, relative to the pedicel (Gewecke, 1967a; Gewecke, 1974). The campaniform sensillum, a phasic-tonic receptor, is sensitive to the lateral deviation of the flagellum relative to the pedicel whereas the JOs are phasic receptors sensitive to the frequency of flagellar vibration. Flight velocity is apparently encoded by the JOs in the differential activation of scolopideal sensilla across the JOs, which varies based on the position of the pedicellar–funicular joint (Gewecke, 1974).

As *D. melanogaster* may lack the pedicellar campaniform sensillum present in *C. erythrocephala* (Miller, 1950) (D. Eberl, personal communication), this leaves the JO as the only likely mechanoreceptor sensitive to relative motion at the

pedicellar–funicular joint. Thus, *D. melanogaster* would seem to be an ideal system in which to test the contributions of the JOs to wind sensation, although morphological data in the odonate *Orthetrum cancellatum* also suggest a unitary contribution of the JOs to velocity control (Gewecke et al., 1974; Gewecke and Odendahl, 2004).

By fixing the funiculus and pedicel relative to each other, we have shown that the JOs seem to play an essential role in the detection of wind direction. Flies bilaterally deafferentated at the JO were unable to orient upwind in a 0.2 m s^{-1} wind, although they did so successfully at 1.0 m s^{-1}. These responses were similar to those of dead, winged flies, implying that at elevated wind speed they could be explained largely by passive mechanisms, with perhaps a small contribution from other mechanoreceptors, or a visually mediated response. In flies with a single antenna glued, the response declined substantially at 0.2 m s^{-1} (compared to the control group) as flies failed to orient significantly above baseline, though responses in flies with the right antenna glued were nearly significant.

These results suggest a marked, but incomplete, loss of orienting ability in unilaterally deafferentated flies and thus provide evidence against a model of wind detection that is strictly dependent on input from both antennae. Indeed, orientation improved by 73% at 0.2 m s^{-1} with the restoration of mechanosensory input from a single antenna, suggesting an ability to detect wind direction with a single chordotonal organ. Further, the data indicate partial contributions from both antennae as orientation improved by an additional 52% and 20% with mechanosensory input from a second antenna, at 0.2 and 1.0 m s^{-1}, respectively. However, the data do not preclude the involvement of an additional mechanism, based on an interantennal comparison of deflections, in determining wind direction. The bias that we observed in turn direction among unilaterally glued flies may hint at such a possibility. We attempted to address the role of the JOs genetically via experiments on the chordotonal mutant *Beethoven* (Eberl et al., 2000), but this fly was completely unable to fly in our paradigm.

In the present experiments, we have shown that the superposition of wind on an expanding visual stimulus is capable of reshaping the orientation response to the extent that it can completely suppress expansion avoidance under the appropriate conditions. Upwind orientation generally increased with wind velocity across expansion rates, and decreased with expansion rate across wind velocities, although the contributions of the two stimuli are more difficult to interpret when they favored overlapping orientations. It thus appears as though wind fixation can be described as a relatively simple multivariate function of wind velocity, expansion rate and location of the FOC. Moderate misalignment between the FOC and the upwind direction allows for a compromise orientation, whereas flies generally choose between one of the attractive stimuli as they become diametrically opposed.

The partial regression coefficients from our multivariate analysis suggest that during free-flight the expansion avoidance response may be largely suppressed by a mechanically induced preference for forward flight. For example, following contact with a plume of attractive odorant, flies routinely achieve air speeds of 0.6 m s^{-1}. If the resulting rate of visual expansion was in the range of velocities examined in this study, it would not be at all difficult to explain how a fly is able to rapidly proceed upwind under these conditions. These findings suggest a working hypothesis for a flight control strategy that emerges from the interaction of the upwind orientation and expansion avoidance responses. The wind-mediated orientation inhibits expansion avoidance during forward flight; however, the upwind response must give way to expansion-avoiding turns when the expansion becomes large, presumably due to the visual cues associated with imminent collisions.

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